AD _____

LASSA FEVER IMMUNE PLASMA

ANNUAL REPORT

John D. Frame

July 31, 1989

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick Maryland 21701-5012

Contract No. DAMD17-85-C-5189

Columbia University College of Physicians and Surgeons
630 W. 168th Street
New York, N.Y. 10032

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Columbia University, College of Physicians and Surgeons; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): 630 West 168th Street, New York, N.Y. 10032	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: U.S.Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable): SGRD-RMI-S	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-85-C-5189
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 63750; PROJECT NO. 3M463 750D809; TASK NO. AS; WORK UNIT ACCESSION NO 049

11 TITLE (Include Security Classification): (U) Lassa Fever Immune Plasma

12. PERSONAL AUTHOR(S): John D. Frame

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 1Jul 88 TO 30Jun89	89-7-31	17

16. SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	13	0606
06	06	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Lassa fever, Immunotherapy, RA 1; Lassa virus, Liberia, (JES); Plasma, human.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the year the Field Investigator, Mr. J.E. Yalley-Ogunro, emigrated to the United States; at the US Army Medical Research Institute of Infectious Diseases, he isolated Lassa virus (LV) from 85 patients whose speciemsn had been accumulating there over an 18 month period. In his absence from Liberia the Clinical Investigator, Dr. Andrew Cole, assumed many of Mr. Yalley-Ogunro's responsibilities. However, serological testing for LV antibodies by the indirect fluorescent antibody test was suspended; the technologist chosen to perform this test proved to be personally unreliable, and was dismissed.

The loss of another technologist, that at Phebe Hospital (PH), coincided with a marked increase in the number of Lassa fever cases in the institution, and resulted in state of disarray in the program there. Dr. Cole and the Hospital Director took steps to correct the poor record keeping and it is expected that under the direction of a

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☑ SAME AS RPT ☐ OTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301/663-7325; 22c. OFFICE SYMBOL: SGRD-RMI-S

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

senior staff person in the hospital the problems are being solved.

Plasmapheresis resulted in the collection of 103 LFIP units. Passive immunotherapy with LFIP was used in 84 suspected LF patients at PH; the case fatality was 12% among both confirmed and unconfirmed LF cases.

Summary

The fourth year of investigations of Lassa fever (LF) in Liberia under the extended Contract DAMD17-C-85-5189 began with the departure from Liberia of the Field Investigator and Resident Head, Mr. J.E. Yalley-Ogunro. He had been the key figure of the Liberian program for eight years, and his departure required the transfer of many of his responsibilities to Dr. Andrew Cole, the Clinical Investigator.

An outbreak of LF in the vicinity of one of the field stations, Phebe Hospital (PH), taxed the resources of this institution. It became apparent that record keeping of LF patients, and of plasmapheresis to procure Lassa Fever Immune Plasma (LFIP) were below standard; Dr. Cole and Dr. Walter Gwenigale, Director of PH, corrected the deficiencies. At Curran Lutheran Hospital plasmapheresis and the testing and treatment of patients continued in good order, though the amount of plasmapheresis performed was below the levels of past years, when Mr. Yalley-Ogunro had supervised the procedure directly. In all, 103 LFIP units were obtained during the year.

Upon his arrival at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Mr. Yalley-Ogunro began to attempt virus isolation from specimens that had been accumulating for over a year. He isolated Lassa virus (LV) from 85 patients. In his absence from Liberia serological testing of sera by the indirect fluorescent antibody (IFA) technique lapsed. The technologist who had been expected to fulfill this function proved to be personally unreliable and was dismissed.

Because of the pressure of other research at USAMRIID, determinations of the Log Neutralization Index (LNI) of plasma units were not carried out. Ignorance of the potency of LF units as measured by LNI's and the deficiencies in documentation mentioned above limited the evaluation of the results of the treatment with LFIP of 84 clinically suspected LF cases. The overall mortality rates of both the patients found by virus isolation to have LF and those not so diagnosed were about 12%.

Foreword

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.

Table of Contents

Introduction:

Lassa fever (LF) was first documented in Liberia in an outbreak at Curran Lutheran Hospital (CLH) in 1972 (1). Subsequent surveys of hospitals in all regions of Liberia showed high prevalences of antibodies to Lassa virus (LV) among staff members, notably in Lofa County to the north (2,3). LF was found to be a major cause of febrile illness among patients in hospitals in both Lofa and Bong Counties (4) and to be prevalent in surrounding villages (5). Field stations for LF research have been established in CLH and Kolahun Hospital (KH) in Lofa County, and at Phebe Hospital, Bong County.

Since 1979 research in LF has been conducted under the aegis of the United States Army Medical Institute of Infectious Diseases (USAMRIID) which has supplied logistical and virological support, and specific reagents for the serodiagnosis of LF. Investigations in Liberia have heretofore been centered at the Liberian Institute for Biomedical Research. A fluorescence microscope there has made possible the monitoring of LF by means of the indirect fluorescent antibody (IFA) technique.

A major part of the work in Liberia has been the procuring of Lassa Fever Immune Plasma (LFIP) from convalescents. In accordance with the results of Dr. Peter Jahrling at USAMRIID, plasma donors are recalled about 6 months after their recovery from acute illness, and requested to donate plasma by plasmapheresis. Dr. Jahrling has found that it requires this length of time for the titers of protective antibody, measured by the Log Neutralization Index (LNI), to reach levels high enough to warrant use in the treatment of LF (6). LFIP is being used regularly in Liberian hospitals in the treatment of LF. Most of the plasma obtained, however, has been brought to the USAMRIID for the production of Lassa-specific immune globulin for projected investigations of its value in treatment.

It has been difficult to demonstrate the usefulness of LFIP in the treatment of LF, in spite of a number of anecdotal reports of its value (7,8,9,10). This has been due in part to the varying neutralizing capacities of the LFIP units, as well as the varying degree of morbidity in LF cases. It is anticipated that in the near future investigations of the value of Lassa Fever Immune Globulin (LFIG) will be possible. Immune globulin from plasma pools will likely be rather uniform in potency. Furthermore, it is planned to use changes in virus titer during treatment rather than the clinical response as a measure of effectiveness of plasma. The present investigation is probably the last in which LFIP will be the subject of testing, though plasma collection as a source of LFIG will likely continue for some time to come.

Activities

Early In July, 1988, Mr. J.E. Yalley Ogunro, the Field Investigator who had been with the LFIP program since early 1980, emigrated to the United States. Soon after his arrival at USAMRIID he began to attempt virus isolation from sera that had been accumulating since December 1987, and continued this work until he was given new duties at USAMRIID.

With his departure from Liberia Dr. Andrew Cole, the Clinical Investigator, assumed many of the responsibilities that had been Mr. Yalley-Ogunro's. However, testing of patient sera for Lassa virus (LV) antibodies by the indirect fluorescent antibody (IFA) technique could not be carried out. It had been expected that Mr. Adolphus Sienla, a qualified laboratory technologist, would continue serological work. He proved unreliable in his new position, and eventually was dismissed. It is expected that technicians of the Curran Lutheran and Phebe Hospitals will be instructed in the IFA technique by Ms. Betsy Brotman of VILAB II of the New York Blood Center, stationed at the LIBR.

Before his departure from Liberia Mr. Yalley-Ogunro had taught the technique of the enzyme-linked immunosorbent assay (ELISA) to the technologists at CLH. However, evaluation of the results of experiments (10) he had performed during the previous year indicated that the antigen-capture ELISA, a potential means for the early diagnosis of LF, was not yet ready for use in the field.

Plasmapheresis of LFIP units continued at both CLH and PH: it was performed by the laboratory staffs of the hospitals. Diagnosis of suspected cases of LF was made by the clinical staffs of the hospitals, and specimens for diagnosis collected in both institutions. Plasma units and serological specimens were transported to the LIBR by Dr. Cole, and forwarded to USAMRIID with the help of VILAB II.

The Principal Investigator, John D. Frame, M.D. visited the sites in Liberia in January and April. He determined that at CLH the local laboratory staff was carrying out the obtaining of sera from patients and plasmapheresis in an exemplary fashion under the guidance of Ms. Jinny Buck, laboratory instructor, and Mr. David Dorborson, laboratory Chief. Records and inventory were in good condition.

On the April visit to PH it became apparent that without the guidance of Mr. Yalley-Ogunro, and in the face of an apparent LF outbreak in the region the program at PH was in a state of disarray. This was exacerbated by the resignation from the hospital of the technologist who had coordinated both the testing of patients and plasmapheresis. Prompt re-ordering of supplies by notification of the Clinical or Principal Investigator had not been done when the magnitude of the outbreak became apparent; identification of patient sera was incomplete, patient and

financial record keeping was poor. The state of the program there was brought to the attention of the Hospital Director, Dr. Walter Gwenigale, who subsequently appointed a senior member of his staff to be responsible for the LF program at the hospital. Dr. Andrew Cole returned to PH on several occasions in the coming months, and was able to assist in correcting major deficiencies.

Plasmapheresis

During the year 103 LFIP units were obtained from convalescents from LF. A number were forwarded to USAMRIID. No record of the units received at USAMRIID has been sent the Principal Investigator. More than the usual number of units were used in Liberia, because of the outbreak of suspected LF at PH. No report of the LNI's of the specimens sent to USAARMIID have been reported since December 1987; testing has apparently been limited by the shortage of qualified laboratory personnel at USAMRIID.

Lassa Fever cases

Both serological and virological techniques are used in the diagnosis of LF. Serodiagnosis depends upon seroconversion or a four-fold rise in LV antibody titers in serial specimens, as determined by the IFA technique. In some cases the initial specimen is obtained from the patient late in the course of the illness when the IFA titer is already high; in others, only one specimen may be obtained, and comparison of titers between sera is not possible. If a single specimen only is obtained and the IFA titer is 1:64 or higher, the patient is classsified as Possible LF (PLF).

We have demonstrated earlier that when virus isolation is performed the diagnosis of LF may be made in patients among whom it is missed by serodiagnosis (12). Similarly, virus isolation may not be possible if the specimen is obtained after the viremic stage, or if refrigeraton is inadequate to maintain virus activity.

With the absence of Mr. Yalley-Ogunro from Liberia no serological testing was carried out during the year. Working at USAMRIID, he was able to attempt virus isolation, not only on patients seen during the year, but also on a backlog of specimens of patients seen earlier, but tested only by serodiagnosis. The results are given in Table 2 in the Appendix. LV was isolated from 85 of 428 patients tested.

In both CLH and PH a number of specimens were tested by virus isolation which were reported last year on the basis of the results of serodiagnosis only. In addition, in each hospital a number of patients treated since May, 1988, were tested; only virus isolation was performed on them. The incidence of LF among the fever patients in CLH, 15 virus-positive out of 252 or 10.6%, was of the same order as in past years. In PH, on the other hand, the incidence of LF among cases of fever was higher in both years than formerly, and about twice as high in the last seven months of the

survey than in the previous 6 months. At PH LV was isolated from 58 of 210, or 27.6% of patients

The results are in keeping with the clinical impression at PH, that the staff was seeing many more LF cases than in the past, that they were dealing with an outbreak of LF. In Table 2 this is even more apparent, when the cases are evaluated which were seen over a limited period in which virus isolation was attempted on all patient specimens.

The laboratory diagnosis of LF was made at PH about three times as often in May through September, 1988, as it was in the same period in 1986. (Tests of statistical significance are not appropriate, as in both groups an uncertain number of patient sera were lost and not tested). The incidence of LF at CLH appeared to be lower in the more recent period, again in line with the clinical impression of the staff there, though the numbers are too small to permit valid comparisons.

Passive Immunotherapy

During the last 18 months 84 patients were treated with LFIP units at PH (Appendix, Table 4), and 10 died. As in the past, in the absence of laboratory tests suitable for early diagnosis of LF, treatment was instituted on the basis of the physicians' clinical judgment; in some patients who were treated the diagnosis of LF could not be confirmed. Furthermore, as noted above, serodiagnosis was not attempted during the year. Inasmuch as LNI's of the LFIP units administered were not determined, no evaluation of their potency could be made. Records were not maintained adequately, so that plasma was administered to some patients whose diagnostic category could not be determined.

In any case, the rate of survival of all the patients, whether viremic or not, was essentially the same, about 12%.

Conclusion

The year was one of adjustment to a change of leadership and other personnel in the LFIP program in Liberia. It appears that the Clinical Investigator and workers in the hospitals are becoming familiar with their new responsibilities.

From a technical point of view it is increasingly apparent that means for early laboratory diagnosis of LF in the field are needed to conserve supplies of LFIP for use in LF patients. It is also clear that determinations of the potency of LFIP by measurement of LNI's is required if the usefulness of passive immunotherapy is to be established.

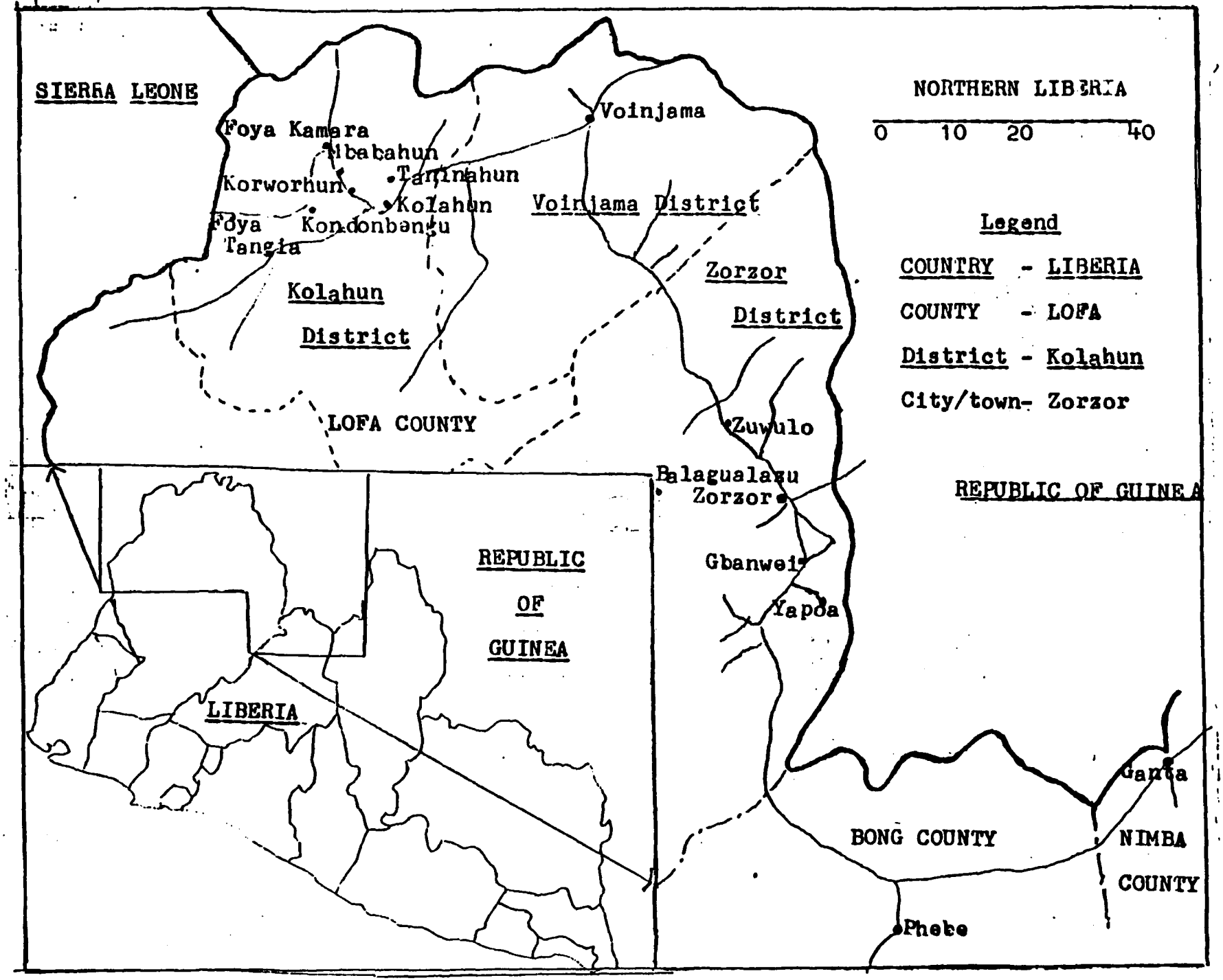
NORTHERN LIBERIA
0 10 20 40
SIERRA LEONE
Foya Kamara
Ibabahun
Korworhun
Tamnahun
Kolahun
Foya
Tangia
Kondonbangu
Voinjama
Voinjama District
Zorzor
District
Kolahun
District
LOFA COUNTY
Zuwulo
Balagualazu
Zorzor
REPUBLIC OF GUINEA
Gbanwei
Yapoa
REPUBLIC
OF
GUINEA
LIBERIA
Ganta
BONG COUNTY
NIMBA
COUNTY
Phebe
Legend
COUNTRY - LIBERIA
COUNTY - LOFA
District - Kolahun
City/town- Zorzor

Table 1. Lassa Fever Immune Plasma Units collected July 1, 1988 - June 30, 1989.

Donor	Date of Donation	IFA titer*	LNI# Jos	LNI# Mac	Number of Units Collected	Number of Units to USAMRIID
Phebe Hospital						
GaAb	4/7/89				2	
	6/22/89				2	
EmBa	6/6/89				2	
GoBa	3/16/89				2	
CoBo	3/5/89				2	
ToBo	3/5/89				2	
JaDa	7/25/84		1.4			
	6/15/88	32				
	5/5/89				2	
	5/29/89				2	
CaFa	4/19/89				2	
MaFa	12/5/85		0.3	0.4		
	4/24/89				2	
	6/22/89				2	
JaFa	12/5/88				2	
ZiHo	5/17/89				2	
MoKe	2/22/89				2	
	3/10/89				2	
	4/18/89				2	
AnKo	6/15/88	4				
	1/16/89				2	
	6/6/89				2	
JoKo	4/24/87	4	0.1	0.4		
	1/2/89				2	
	3/3/89				2	
SoKw	6/16/88	8				
	1/23/89				2	
MaKe	4/11/89(?)				2	
	4/17/89				2	

Table 1, cont.

Donor	Date of Donation	IFA titer*	LNI# Jos	LNI# Mac	Number of Units Collected	Number of Units to USAMRIID
Phebe Hospital, cont.						
JaMa	6/27/89				2	
YoMc	4/26/89				1	
JoMi	11/10/87		0.6			
	6/5/88	16				
	1/28/89				2	
	4/17/89				2	
MaMi	3/28/89				2	
BeMu	1/6/89				2	
DaMu	6/20/866		0.4			
	1/7/89				2	
	4/17/89				2	
SaPa	6/16/88	4				
	1/6/89				2	
AlSa	12/6/88				2	
	4/12/89				2	
HaTe	3/16/89				2	
	5/5/89				2	
JoTo	6/22/87		32			
	11/10/87			0.4		
	1/19/89				2	
	3/1/89				2	
	5/30/89				2	
MaVo	4/17/89				2	
	5/17/89				2	
	Subtotal, Phebe				81	
Curran						
DaDo	11/30/87		0.4			
	6/7/88	16				
	12/9/88				2	
	2/17/89				2	

Table 1, cont.

Donor	Date of Donation	IFA titer*	LNI# Jos	LNI# Mac	Number of Units Collected	Number of Units to USAMRIID
JoGa	12/15/87		0.4			
	6/8/88	32				
	2/17/89				2	
JoHo	12/1/87		0.9			
	5/7/88	8				
	2/17/89				2	
DaJa	9/11/86		0.4			
	12/9/88				2	
DaKo	3/19/87		3.9+			
	6/7/88	64				
	12/9/88				2	
DaSu	9/10/86		0.5			
	12/9/88				2	
	2/17/89				2	
BeVa	3/19/86		1.0	0.9		
	6/8/88	8				
	12/17/88				2	
	2/17/89				2	
MoWo	12/2/87		1.6			
	6/8/88	32				
	2/17/89				2	
	Subtotal, Curran				22	
	Total				103	

* Recorded as the reciprocal of the Indirect Fluorescent Antibody titer. No IFA titers have been determined since June, 1988. In the case of plasma donors whose plasma was tested previously, the most recently determined titers are included for information.

Jos = Log Neutralization Index (LNI) determinations against the Josiah strain of LV; Mac = LNI's against the Macenta strain. No LNI determinations have been reported by USAMRIID since late 1987. For information purposes the most recent LNI determination is recorded for donors whose LNI's are known.

Table. 2. Incidence of Lassa Fever among febrile patients in selected Liberian hospitals, December 1987 - Spring, 1989

Hospital/ Dates	No. tested	Lassa Fever			Possible LF (High IFA titers)	Total LF and Possible LF (Rate)	Other IFA pos.
		Virus isolation	Seroconversion	Total (Rate)			
Curran							
12/87-5/88	148	15	7	22 (0.154)	8	30 (0.203)	7
6/88-4/89	104	12	*	12 (0.115)	*		
Phebe							
12/87-5/88	128	26	8	34 (0.266)	3	38 (0.296)	9
7/88-2/89	82	32	*	32 (0.451)	*		
Kolahun							
3 & 4/88	6	-		-			

* Not tested

Table 3. Comparison of incidence of Lassa Fever from June to September in 1986 and 1988, Curran and Phebe Hospitals, Liberia.

Hospital/ Dates	No. tested	Lassa Fever		
		Virus isolation	Seroconversion	Total (Rate)
Curran				
5/1/86-9/30/86	52	6	3	9 (0.173)
5/1/88-9/30/88	81	6	-*	6 (0.074)
Phebe				
5/1/86-9/30/88	62	8	-	8 (0.129)
5/1/88 9/30/88	67	23	-*	23 (0.343)

* Tested in May only.

Table 4. The use of Lassa Fever Immune Plasma in febrile patients in Phebe Hospital, Liberia, January 1988 - February 1989

	Number	Recovered	Died
Virus Isolation Attempted			
Patients identified			
Virus isolated	18	16	2
Virus not isolated	17	15	2
Patients not identified	20	18	2
VIrus isolation not attempted	29	25	4
Total	84	74	10

References

1. Monath, TP, Mertens, PE, Patton, R, Moser, CR, Baum, JJ, Pinneo, L, Gary, GW, & Kissling, R. A hospital outbreak of Lassa fever in Zorzor, Liberia, March - April, 1972. Amer J Trop Med Hyg 22: 773-784 (1973)

2. Frame, JD, Casals, J, & Dennis, EA. Lassa virus antibodies in hospital personnel in Western Liberia. Trans Roy Soc Trop Med Hyg 73: 219-224 (1979)

3. Frame, JD, Yalley-Ogunro, JE, & Hanson, AP. Endemic Lassa fever in Liberia. V. Distribution of Lassa virus activity in Liberia: Hospital staff surveys. Trans Roy Soc Trop Med Hyg 78: 761-763 (1984)

4. Frame, JD, Jahrling, PB, Yalley-Ogunro, JE, & Monson, MH. Endemic Lassa fever in Liberia. II. Serological and virologiacl finding in hospital patients. Trans Roy Soc Trop Med Hyg 78:656-660 (1984).

5. Yalley-Ogunro, JE, Frame, JD, & Hanson, AP. Endemic Lassa Fever in Liberia. VI. Village serological surveys for evidence of Lassa virus infection. Trans Roy Soc Trop Med Hyg 78: 764-770 (1984)

6. Jahrling, PB & Peters, CJ. Passive antibody therapy in cynomologus monkeys. Importance of neutralizing antibodies and Lassa virus strain. Infect and Immun 44: 528-533 (1984)

7. Clayton, AJ. Lassa immune serum. Bull W.H.O. 55: 453-439 (1977)

8. Frame, JD, Verbrugge, CP, Gill, RG and Pinneo, L. The use of Lassa fever convalescent plasma in Nigeria. Trans Roy Soc Trop Med Hyg 78: 319-324 (1984)

9. Frame, JD. Annual Summary Report, Lassa Fever Immune Plasma. Trustees of Columbia University in the City of New YOrk. New York, N.Y. July 31, 1987, p. 5.

10. Frame, JD. Annual Summary Report, Lassa Fever Immune Plasma. Trustees of Columbia University in the City of New YOrk. New York, N.Y. July 31, 1986, p. 5.

11. Frame, JD. Annual Summary Report, Lassa Fever Immune Plasma. Trustees of Columbia University in the City of New YOrk. New York, N.Y. July 31, 1988, p. 5.

12. Frame, JD. Annual Summary Report, Lassa Fever Immune Plasma. Trustees of Columbia University in the City of New YOrk. New York, N.Y. July 31, 1983, p. 11.

DISTRIBUTION LIST

5 copies	Commander U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD 21701-5011
1 copies	Commander U.S. Army Medical Research and Development Command ATTN: SGRD-RMI-S Fort Detrick, Frederick, MD 21701-5011
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of the Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100
1 copy	Director Walter Reed Army Institute of Research Walter Reed Army Medical Center ATTN: SGRD-UWZ-C Washington, DC 20307-5100
1 copy	Commander US Army Medical Material Development Activity ATTN: SGRD-UMS-B/W. Ferguson Fort Detrick, Frederick MD 21701-5009
2 copies	Defense Technical Information Center ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145